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EFFECT OF ZEOLITE ON TOXICITY OF AMMONIA IN FRESHWATER SEDIMENTS: IMPLICATIONS FOR TOXICITY IDENTIFICATION EVALUATION PROCEDURES

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Abstract—Techniques for reducing ammonia toxicity in freshwater sediments were investigated as part of a project to develop toxicity identification and evaluation (TIE) procedures for whole sediments. Although ammonia is a natural constituent of freshwater sediments, pollution can lead to ammonia concentrations that are toxic to benthic invertebrates, and ammonia can also contribute to the toxicity of sediments that contain more persistent contaminants. We investigated the use of amendments of a natural zeolite mineral, clinoptilolite, to reduce concentrations of ammonia in sediment pore water. Zeolites have been widely used for removal of ammonia in water treatment and in aqueous TIE procedures. The addition of granulated zeolite to ammonia-spiked sediments reduced pore-water ammonia concentrations and reduced ammonia toxicity to invertebrates. Amendments of 20% zeolite (v/v) reduced ammonia concentrations in pore water by ≥70% in spiked sediments with ammonia concentrations typical of contaminated freshwater sediments. Zeolite amendments reduced toxicity of ammonia-spiked sediments to three taxa of benthic invertebrates (*Hyalella azteca, Lumbriculus variegatus*, and *Chironomus tentans*), despite their widely differing sensitivity to ammonia toxicity. In contrast, zeolite amendments did not reduce acute toxicity of sediments containing high concentrations of cadmium or copper or reduce concentrations of these metals in pore waters. These studies suggest that zeolite amendments, used in conjunction with toxicity tests with sensitive taxa such as *H. azteca*, may be an effective technique for selective reduction of ammonia toxicity in freshwater sediments.

Keywords—Toxicity identification Ammonia Sediment toxicity Zeolite Metals

INTRODUCTION

Ammonia is a natural constituent of aquatic sediments, but high concentrations of ammonia, which occur in sediments affected by pollution from sewage, industrial effluents, and agricultural runoff, can be toxic to sediment-dwelling invertebrates [1]. Although benthic invertebrates are generally more tolerant of high ammonia concentrations than are fish [2], sensitivity to ammonia toxicity varies widely among invertebrate taxa [3]. Ammonia commonly occurs in sediments of industrialized harbors and waterways at concentrations that may be toxic to sensitive benthic invertebrates, such as the amphipod *Hyalella azteca* [4–6]. Several studies have identified ammonia as an important contributor to toxicity in contaminated freshwater sediments [1,7,8]. Uncertainty about the importance of ammonia to toxicity of freshwater sediments, where ammonia often co-occurs with high concentrations of other toxicants such as heavy metals and persistent organic compounds, has led to efforts to develop toxicity identification and evaluation (TIE) procedures for ammonia and other sediment contaminants [9].

Toxicity identification and evaluation procedures identify the cause(s) of toxicity in complex mixtures by selectively reducing the toxicity of specific classes of toxicants [10]. Results of TIE studies can be used to identify sources of toxicants and guide treatment of effluents. Initial efforts to identify sources of toxicity in contaminated sediments have used modifications of TIE procedures developed for aqueous effluents and have assessed changes in pore-water toxicity using toxicity tests with fish and planktonic invertebrates [9]. Ideally, sediment TIE procedures would utilize standardized whole-sediment toxicity tests with benthic invertebrates, which are more realistic indicators of the toxicity of contaminated sediments [11]. However, development of TIE procedures for whole sediments has been limited by the lack of methods for selective reduction of toxicity in whole sediments rather than in isolated pore water.

Our initial attempts to selectively reduce ammonia toxicity in sediments were based on the pH manipulation methodology used for aqueous TIE procedures. Changes in pH shift the equilibrium between the ammonium ion (NH_4^+) and un-ionized ammonia (NH_3^0), which is generally considered to be the more toxic species [2]. An increase in sample toxicity with increased pH provides evidence of ammonia toxicity, because un-ionized ammonia makes up a greater proportion of total ammonia as pH increases in the circumneutral range (pH 6–8) [9]. Our efforts to adjust the pH of whole sediments by addition of mineral acids and bases caused undesirable side effects (e.g., formation of precipitates or evolution of gases), and resulting pH shifts were often temporary. Addition of organic buffers (2-[*N*-morpholino]ethanesulfonic acid [MES], dissociation constant [pK_a] = 6.1; piperazine-*N,N'*-bis[2-hydroxy]propanesulfonic acid [POPSO], pK_a = 7.8; Sigma, St. Louis, MO, USA) did not cause visible precipitates or gas evolution, but produced lesser shifts in pH (0.3–0.5 units) that were insufficient to substantially alter ammonia toxicity. More recently, we have investigated the use of selective sorbents added to whole sediments to reduce concentrations of ammonia in pore water. Other researchers have used selective sorbents in the development of sediment TIE procedures for nonpolar organic compounds [12]

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and cationic metals [13]. This approach, like the equilibrium partitioning approach used for development of sediment quality criteria [14,15], assumes that the toxicity of sediment contaminants is related to concentrations of toxicants in pore water. This assumption has been supported by a recent study of ammonia toxicity in freshwater sediments [16].

The studies reported here investigated the addition of a natural zeolite mineral to whole sediments to selectively reduce pore-water ammonia concentrations and reduce ammonia toxicity to benthic invertebrates. Zeolites have a high exchange capacity for ammonium and other cations because of their unique structure, which consists of an aluminosilicate matrix that forms a large volume of channels filled with water and exchangeable cations [17]. Zeolites have been used previously to confirm ammonia toxicity in aqueous TIE studies with sediment pore water [1]. The abundant zeolite clinoptilolite (typical chemical formula: $Na_6[(AlO_2)_6(SiO_2)_{30}]\cdot 24H_2O$) has a high cation exchange capacity (>2 meq/g) and has been widely used for removal of ammonia in wastewater treatment and aquaculture [18,19]. Clinoptilolite has also been used for removal of metals from wastewater and for immobilization of metals in contaminated soils [17,20] However, some evidence exists that clinoptilolite may preferentially remove ammonium from solutions that also contain heavy metals [17]. The objectives of our studies were to evaluate the use of clinoptilolite amendments to reduce pore-water ammonia concentrations and ammonia toxicity in whole-sediment toxicity tests and to evaluate possible interactions of clinoptilolite with two metals, copper and cadmium.

MATERIALS AND METHODS

Sediment preparation

Ammonia-contaminated test sediments were prepared by spiking both formulated and natural sediments. An uncontaminated natural sediment was collected from Little Dixie Lake, Missouri, USA (4% sand, 47% silt, 49% clay; 2% organic carbon). Formulated sediment was prepared using the following ingredients: silica sand (#1 size; New England Silica, South Windsor, CT, USA), silt–clay mix (ASP 400; Mozel, St. Louis, MO, USA), cellulose (α-cellulose; Sigma/Aldrich, St. Louis, MO, USA), humic acid (Sigma/Aldrich), and powdered dolomite (N. Kemble, personal communication). Equal weights of sand and silt–clay mix were mixed, and additional ingredients were added to produce desired concentrations in the dry mixture: 2% total organic carbon (cellulose), 0.5% dolomite, and 10 mg/kg humic acid. The formulation had a particle size distribution of 52% sand, 33% silt, and 15% clay. The dry mixture was mixed on a rolling mill for 1 h then mixed with test water. Test water used for all studies was well water diluted 1:1 with deionized water (pH = 8.0, total alkalinity = 1.3 mM, total hardness = 1.7 mM). Ammonia spike solutions were prepared with ammonium chloride in test water. Spike solutions were added directly to dry formulated sediment mix at a ratio of two volumes solution to one volume dry sediment. Spike solutions were mixed into wet Little Dixie sediments at a 2:1 ratio (v/v) and the sediments were sieved (30-mesh sieve; 0.5-mm pore diameter) to remove coarse particles and native benthic organisms. Control sediments for ammonia spiking studies were mixed with ammonia-free test water. Ammonia-spiked sediments were held at least 2 d before bioassays or amendments with zeolite.

Sediments contaminated with cadmium (Cd) and copper (Cu) were obtained from the U.S. Environmental Protection Agency, Midcontinent Ecology Division (U.S. EPA-MED, Duluth, MN). Cadmium-spiked sediments were prepared at U.S. EPA-MED from sediments from West Bearskin Lake, Minnesota, USA [21]. Sediments were spiked with a small volume of a cadmium spike solution ($CdCl_2$ in deionized water, neutralized with NaOH) to produce a concentration of 660 mg Cd/kg (wet weight). Sediments and spike solutions were mixed thoroughly and refrigerated (4°C) for 10 d before testing. Sediments contaminated with copper from mine tailings were collected from the Keweenaw Waterway, Michigan, USA [22]. Keweenaw sediments were mixed with test water (1:2 ratio) and stored for 2 weeks at 4°C before use.

Zeolite amendments were prepared from clinoptilolite granules (Aquatic Eco-Systems, Apopka, FL, USA), which were ground to sand- and smaller-sized particles (56% sand, 23% silt, 21% clay) and wetted with excess test water. The mixture was allowed to settle for 24 h, overlying water was decanted, and the resulting zeolite slurry was added to test sediments 2 d before bioassays. All zeolite amendments are reported as nominal additions on a volume basis. For example, a 10% (v/v) zeolite treatment refers to addition of 100 ml of zeolite slurry to 1 liter of wet sediment, which produced a mixture containing 9.1% zeolite. Most sediments used as controls for zeolite treatments received no amendments or manipulations, but Keweenaw sediments not amended with zeolite received an amendment of an equal volume (20% [v/v]) of silica sand.

Toxicity tests and chemical analyses

Whole-sediment toxicity tests were conducted with three test organisms: amphipods (*H. azteca*), oligochaetes (*Lumbriculus variegatus*), and midges (*Chironomus tentans*) [23]. Tests were conducted at 23°C (±1°C), with temperature and dissolved oxygen of overlying water monitored daily and food (yeast–ryegrass–trout food suspension [23]) added on days 0 and 2. Four replicate test chambers (300-ml beakers containing 100 ml of sediment, 175 ml of water, and 10 test organisms) were used for each treatment group. Most tests were conducted for 96 h under static conditions, as preliminary studies demonstrated that 96-h tests were sufficient to document lethality due to ammonia. Static tests were used to avoid dilution of ammonia concentrations by replacement of overlying water. One 10-day test with *C. tentans* was conducted to evaluate the relative sensitivity of chronic survival and growth endpoints. This test was conducted with replacement of overlying water (1 volume-replacement/d) and daily feeding.

Ammonia concentrations and other water quality characteristics were measured in sediment pore water on days 0 and 4 and in overlying water on day 2. Pore-water samples were prepared by centrifugation of 100 to 200 ml of sediment for 20 min at 3,000 rpm. Total ammonia, pH, and hardness were determined in unfiltered pore-water samples. Total ammonia was analyzed by ion-selective electrode [24] and concentrations of un-ionized ammonia were calculated from total ammonia using published coefficients to correct for effects of pH and temperature [25]. Samples of dissolved metals were prepared by filtering raw pore water through 0.45-μm polycarbonate membrane filters and were acidified with nitric acid (to 1% [v/v]). Samples for cadmium analysis were treated with a $Mg(NO_3)_2/MgPO_4$ matrix modifier. Metal analyses were conducted by graphite furnace atomic absorption spectrophotometry with L'vov platform and Zeeman background correction.

Data from toxicity tests were analyzed to characterize concentration–response curves for ammonia in pore waters of test

Table 1. Acute toxicity of ammonia-spiked formulated sediments and natural sediments (Little Dixie Lake, MO, USA) to aquatic invertebrates. Median lethal concentration (LC50) for 96-h tests and 95% confidence limits calculated from pore-water ammonia concentrations by probit or trimmed Spearman–Karber methods

	Ammonia LC50 (95% CI), mg N/L			
	Formulated sediment		Natural sediment	
Species	Un-ionized	Total	Un-ionized	Total
Amphipod (*Hyalella azteca*)	1.8 (1.4–2.2)	126 (95–167)	0.16 (0.14–0.19)	117 (97–143)
Oligochaete (*Lumbriculus variegatus*)	3.2 (3.1–3.22)	286 (274–300)	0.29 (0.18–0.48)	302 (162–566)
Midge (*Chironomus tentans*)	5.6 (—)	564 (—)	0.53 (0.45–0.63)	430 (363–509)

sediments and to evaluate differences in toxicity resulting from zeolite amendments. Median lethal concentrations (LC50s) and 95% confidence intervals were calculated with the GWBASIC program, LC50, available from U.S. EPA-MED (T. Norberg-King). The trimmed Spearman–Karber method [26] was used to estimate LC50s and confidence intervals if data were not suitable for probit analysis. Analyses for effects of zeolite amendments on invertebrate survival and growth were made by analysis of variance (ANOVA). If ANOVA indicated significant effects of zeolite, or a significant interaction of zeolite and toxicant (ammonia or metal), differences between means for individual treatments were examined by *t* tests (to compare two means) or Duncan's multiple-range test (to compare more than two means) [27].

RESULTS AND DISCUSSION

Toxicity of ammonia-spiked sediments

The three invertebrates tested differed widely in their sensitivity to ammonia. Toxicity tests with spiked formulated and natural (Little Dixie) sediments indicated that *H. azteca* (amphipod) was most sensitive to pore-water ammonia, *L. variegatus* (oligochaete) was intermediate, and *C. tentans* (midge) was least sensitive (Table 1). Sensitivities of these three taxa to ammonia were similar in both sediment types when expressed in terms of LC50 of total ammonia in pore water. Lethal concentrations for un-ionized ammonia differed by an order of magnitude between sediment types, with toxicity occurring at lower concentrations of un-ionized ammonia in the natural sediment. Ammonia toxicity to amphipods, expressed as either total ammonia or un-ionized ammonia, was similar to results of other 96-h tests at comparable pH and hardness [4]. Lethal concentrations of total ammonia to oligochaetes and midges in 96-h tests were two to four times greater than values reported for 10-d tests (*L. variegatus* LC50 = 75 mg/L, *C. tentans* LC50 = 233 mg/L [28]).

Our studies did not find differences in lethal concentrations of total ammonia in pore water between Little Dixie sediment and formulated sediment for any of the species tested, despite substantial differences in pore-water pH between the two sediments. Pore waters of Little Dixie sediments had a pH approximately one pH unit lower than that in pore waters of formulated sediments, across the full range of ammonia concentrations studied (Table 2). Pore waters from ammonia-spiked sediments also had more acidic pH and greater hardness than pore waters from unspiked sediments, apparently due to displacement of hydrogen ions and hardness cations (calcium

Table 2. Effect of zeolite amendments on ammonia concentrations and other characteristics of pore water from ammonia-spiked formulated and natural (Little Dixie Lake, MO, USA) sediments. Values are means of two measurements

			Pore-water characteristics		
Sediment type	Ammonia spike (mg N/L)	Zeolite amendment (% v/v)	Total ammonia (mg N/L)	pH	Hardness (mM)
Formulated	0	0	2.0	7.83	2.2
	0	10	0.2	7.64	4.9
	500	0	299	7.39	4.9
	500	10	52	7.37	10.9
	1,000	0	564	7.29	6.9
	1,000	10	165	7.40	15.4
Natural	0	0	2.4	6.90	1.4
	0	10	0.4	6.78	1.3
	0	20	0.5	6.67	1.5
	750	0	203	6.77	5.4
	750	10	83	6.47	4.5
	750	20	51	6.68	6.5
	2,000	0	783	6.50	8.8
	2,000	10	407	6.49	16.2
	2,000	20	224	6.53	17.6
	3,000	0	730	6.74	6.0
	3,000	10	460	6.68	15.9
	3,000	20	318	6.58	21.9

and magnesium) on sediment exchange sites by ammonium. The apparent lack of pH-dependence of ammonia toxicity is contrary to the common assumption that ammonia toxicity is predominantly caused by un-ionized ammonia [2]. Differences in pH between the two sediment types correspond to a 10-fold difference in the un-ionized ammonia fraction, from 0.15% of total ammonia in Little Dixie sediment (pH approx. 6.5 at LC50) to 1.54% in the formulated sediment (pH 7.5 at LC50) [25]. Some studies of associations between ammonia toxicity and pH have described ammonia toxicity to fish and invertebrates in terms of contributions from both ammonium ion and un-ionized ammonia [29]. Ammonia toxicity to *L. variegatus* and, to a lesser extent, *C. tentans* was found to be pH-dependent in soft water, consistent with toxicity of un-ionized ammonia [4]. However, ammonia toxicity to *H. azteca* has been described as independent of pH, indicating lesser contribution of un-ionized ammonia, in soft water (hardness <0.5 mM) but not in hard water [4,30]. However, a recent study found that sodium and potassium, not calcium and magnesium, reduce toxicity of ammonium to *H. azteca* and that ammonia toxicity was pH-dependent only in waters with high concentrations of sodium (≥1 mM) and potassium (≥0.1 mM) [31]. These findings suggest that the apparent predominance of toxicity from ammonium ions in our studies is related to the low concentrations of potassium (0.04 mM) and sodium (0.6 mM) in the test water. Based on these results, total ammonia concentrations in pore water were used to represent ammonia exposure in the current study.

Effects of zeolite on pore waters of ammonia-spiked sediments

Amendment of ammonia-spiked sediments with zeolite substantially reduced ammonia concentrations in pore water. Addition of 10% zeolite (v/v) to ammonia-spiked formulated sediment reduced pore-water ammonia concentrations by more than 70% during 96-h toxicity tests, relative to sediments without zeolite (Table 2). The same rate of zeolite addition resulted in lesser reductions of pore-water ammonia concentrations (less than 60%) in ammonia-spiked Little Dixie sediment. However, addition of 20% zeolite to Little Dixie sediments spiked with total ammonia up to 2,000 mgN/L resulted in greater reductions of pore-water ammonia, comparable to those measured in formulated sediments receiving 10% zeolite.

The effectiveness of zeolite amendments was apparently more closely related to total ammonia concentrations (pore-water + sorbed) in the spiked sediments than to concentrations in pore water. Pore-water ammonia concentrations after the 10% zeolite treatment were similar in the two sediments, at comparable spike concentrations, despite substantial differences in pore-water ammonia concentrations before treatment (Fig. 1). The slope of the linear regression between ammonia spike concentrations and pore-water ammonia concentrations after the 10% zeolite treatment suggests that the treatment reduced aqueous ammonia concentration by about 76% relative to the spike concentration, regardless of sediment type (r^2 = 0.95, slope = 0.24). This result suggests that the net effectiveness of zeolite amendments for reduction of pore-water ammonia concentrations may vary among field-collected sediments that have similar ammonia concentrations in pore water, but different total ammonia concentrations.

Addition of zeolite amendments to ammonia-spiked sediments generally resulted in greater pore-water hardness, but had little effect on pore-water pH (Table 2). Addition of zeolite

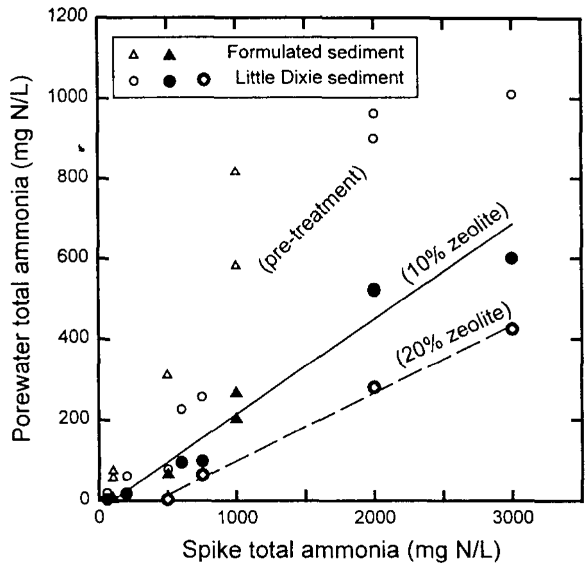

Fig. 1. Effect of zeolite amendments on ammonia concentrations in pore water of formulated sediments (triangles) and natural sediments (circles) spiked with ammonia. Small, open symbols indicate pore-water concentrations before zeolite addition, large symbols indicate concentrations after zeolite treatments (filled = 10% zeolite, open = 20% zeolite). Lines indicate significant linear regressions (solid = 10% zeolite, dashed = 20% zeolite).

to sediments with high pore-water ammonia concentrations (>200 mg N/L) produced very high hardness (>10 mM), due to displacement of calcium and/or magnesium on zeolite exchange sites by ammonium. Hardness cations were apparently the predominant exchange cations in the current studies because calcium and magnesium make up 75% of the molar concentration of cations in the test water. Lesser shifts in hardness occurred in pore waters of control (unspiked) sediments after zeolite additions, suggesting that zeolites would have less effect on the ionic balance of pore waters of natural sediments than on those of ammonia-spiked sediments. Zeolite amendments could serve as either a source or sink for various cations, depending on the characteristics of the sediment and on the treatment of the zeolite before addition. Major changes in concentrations of cations could increase or decrease the toxicity of ammonium ion [31], especially in waters of low ionic strength, although such undesired shifts in cationic composition could be minimized by pretreating zeolites with water that matches the ionic composition of sediment pore waters.

Effects of zeolite on toxicity of ammonia-spiked sediments

Amendment of ammonia-spiked formulated sediments with 10% zeolite reduced the toxicity of these sediments to *H. azteca* and *L. variegatus*. The toxicity of ammonia-spiked sediment to *H. azteca* (100% mortality at both levels of ammonia tested) was significantly reduced by the 10% zeolite treatment (ANOVA and *t* tests; Fig. 2a). Zeolite treatments also significantly reduced ammonia toxicity to *L. variegatus* (ANOVA and *t* tests; Fig. 2b). *Lumbriculus variegatus* showed a more graded response to ammonia-spiked sediments, with partial mortality at the low ammonia concentration and complete mortality at the high concentration. Addition of zeolite eliminated ammonia toxicity at both ammonia levels. The greater sensitivity of *H. azteca* to ammonia was indicated by lower survival in all combinations of ammonia and zeolite, except at the

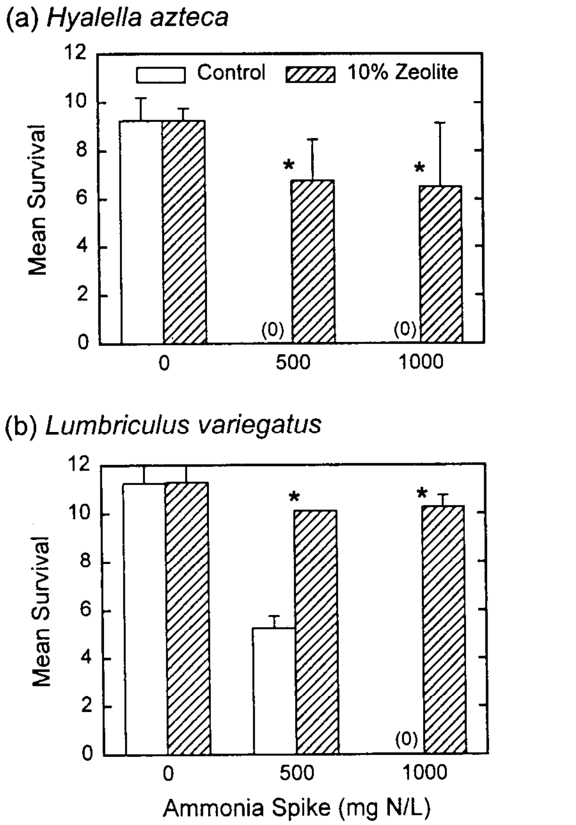

Fig. 2. Effect of zeolite amendments on survival of amphipods (*Hyalella azteca*) and oligochaetes (*Lumbriculus variegatus*) in ammonia-spiked formulated sediment. Bars represent mean survival, with standard deviations (four replicates). Asterisks indicate significant differences between zeolite treatment and control (*t* tests). (**a**) *Hyalella azteca*; (**b**) *L. variegatus*.

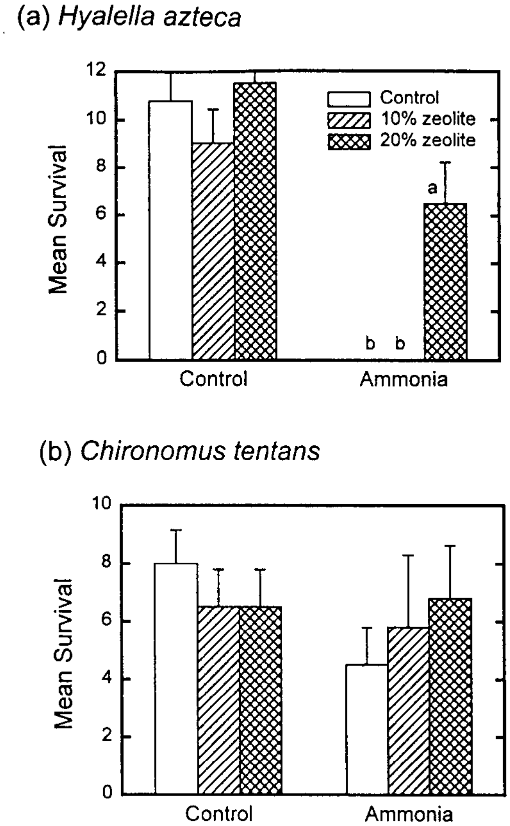

Fig. 3. Effect of zeolite on survival of amphipods (*Hyalella azteca*), and midges (*Chironomus tentans*) in Little Dixie sediments spiked with ammonia. Bars represent mean survival, with standard deviations (four replicates). Bars labeled with different letters indicate significant difference among zeolite treatments (Duncan's test). (**a**) *Hyalella azteca*; (**b**) *C. tentans*.

highest ammonia level (without zeolite), which caused 100% mortality of both taxa.

Addition of a greater amount of zeolite (20%) was required to produce significant reduction in toxicity of ammonia-spiked Little Dixie sediments. Survival of *H. azteca* in ammonia-spiked Little Dixie sediment differed significantly among zeolite treatments, with 100% mortality in the positive control (spike = 2,000 mg N/L) and the 10% zeolite treatment and 65% survival in the 20% zeolite treatment (ANOVA and Duncan's test; Fig. 3a). Results of a 10-d exposure of *C. tentans* to ammonia-spiked Little Dixie sediments (spike = 3,000 mg N/L) provided less-conclusive evidence of the effectiveness of zeolite. The ammonia-spiked sediments caused significant reduction in midge survival (ANOVA; Fig. 3b), although growth of surviving larvae was not affected (ANOVA; data not shown). The effect of ammonia on midge survival was evident only in the sediments without zeolite, where survival was about one half of that in controls. Although the zeolite treatment did not have a significant effect on midge survival, a trend for greater survival at greater zeolite levels was apparent in the ammonia-spiked sediment.

These results indicate that *H. azteca* is a suitable test organism for detecting changes in ammonia toxicity in sediment TIE studies. All ammonia-spiked sediments with pore-water ammonia concentrations of 300 mg N/L or greater caused 100% mortality of *H. azteca* in 96-h static tests, but amendment of these sediments with zeolite increased amphipod survival to 50% or more. Zeolite amendments did not significantly affect survival of any of the test organisms (ANOVA; Figs. 2 and 3). Although the oligochaete and midge taxa tested also provided evidence of reduced ammonia toxicity in zeolite treatments, these taxa are less appropriate test organisms for ammonia TIE studies than *H. azteca* because they are substantially less sensitive to ammonia toxicity.

Effects of zeolite on metal-contaminated sediments

Zeolite amendments did not reduce the toxicity or pore-water cadmium concentrations of cadmium-spiked West Bearskin Lake sediment (Fig. 4a). The cadmium-spiked sediment caused 100% mortality of *H. azteca* during 96-h tests with or without the addition of 20% zeolite. Pore-water cadmium concentrations were nearly identical in cadmium-spiked sediments with or without zeolite amendments, although pore-water cadmium decreased during the course of the study in sediments from both treatments (Table 3). These decreases in pore-water cadmium are similar to those observed in previous studies with cadmium-spiked West Bearskin Lake sediments (E. Leonard, personal communication). Survival was reduced significantly by the zeolite amendment in the control sediment (*t* test). This was the only significant adverse effect of zeolite observed in any of tests conducted during this study.

Zeolite amendments also did not decrease the toxicity of copper-contaminated Keweenaw sediments. The Keweenaw sediments caused greater than 80% mortality of *H. azteca* during the 4-d test, and survival was not increased by the addition of 20% zeolite amendment (ANOVA and *t* tests; Fig.

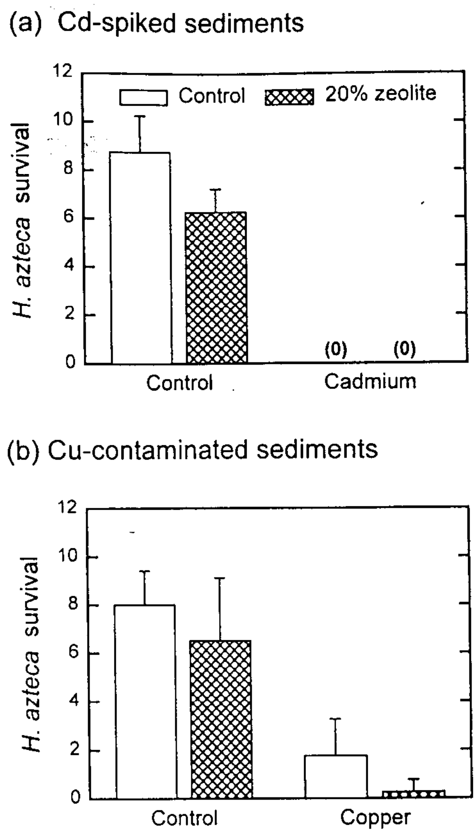

Fig. 4. Effects of zeolite amendments on survival of amphipods (*Hyalella azteca*) in metal-contaminated sediments. Bars represent mean survival, with standard deviations (four replicates). Bars labeled with asterisks indicate significant difference between zeolite treatment and control (*t* test). (**a**) Cadmium-spiked sediment from West Bearskin Lake, Minnesota, USA; (**b**) copper-contaminated sediment from Keweenaw Waterway, Michigan, USA.

4b). Survival was slightly lower in the zeolite treatment in both sediments, although this difference was not significant. Copper concentrations in pore water were similar in Keweenaw sediments with and without zeolite at the beginning of the study (2 d after zeolite addition), but increased more rapidly during the course of the study in untreated sediments than in zeolite-treated sediments (Table 3). These increases in pore-water copper in the Keweenaw sediments may be a response to the manipulation of these sediments before the test, although a similar increase in pore-water copper concentrations was also noted in a previous study with Keweenaw sediments [32].

Increased hardness following zeolite treatments, such as that measured in ammonia-spiked sediments (Table 2), could reduce the toxicity of metals [33] and thus reduce the specificity of the zeolite treatment. However, zeolite amendments had less influence on pore-water pH and hardness in the metal-contaminated sediments than in ammonia-spiked sediments (Table 3). Although addition of 20% zeolite caused a moderate increase in hardness in the cadmium-spiked sediment, zeolite amendments had little or no effect on the unspiked natural sediments (West Bearskin and Keweenaw). These limited data suggest that increased hardness is not a universal result of the zeolite treatment.

The lesser effect of zeolite amendments on toxicity and pore-water composition of metal-contaminated sediments, compared to the effects in ammonia-spiked sediments, suggest that copper and cadmium are exchanged more slowly than ammonium by ion-exchange sites on clinoptilolite [34]. Clinoptilolite amendments caused substantial reductions in total ammonia concentrations in pore water within 2 d, but zeolite amendments did not reduce pore-water concentrations of either cadmium or copper in the 6 d between zeolite addition and the end of the toxicity tests. The greatest effect associated with zeolite amendments was the slower increase in copper concentrations in pore waters of Keweenaw sediments, which resulted in a 50% difference between the control and the zeolite treatment at the end of the test. These results are consistent with previous studies of cation exchange by clinoptilolite. The initial exchange of ammonia by clinoptilolite is especially rapid, with exchange of one third of the equilibrium capacity occurring in the first 10 min [19]. The exchange of ammonium to clinoptilolite was found to be more rapid than exchange of copper or cadmium ions, both in studies with single cations and in studies with mixtures of ammonia and metals [17].

CONCLUSIONS

Amendment of ammonia-spiked sediments with a natural zeolite mineral, clinoptilolite, reduced pore-water ammonia concentrations and reduced toxicity of ammonia-spiked freshwater sediments to benthic invertebrates. Zeolite amendments reduce ammonia toxicity by removing ammonium ions from solution in pore water to zeolite cation exchange sites. The resulting decrease in total ammonia concentrations in pore water should be effective for reducing toxicity of both am-

Table 3. Effect of zeolite amendments on concentrations of metals and other constituents of pore water from metal-contaminated sediments. Hardness and pH are means of two measurements, metal concentrations are individual measurements (day 0, day 4)

Sediment type	Zeolite (% v/v)	Pore-water characteristics: pH	Hardness (mM)	Cd (μg/L)	Cu (μg/L)
Study 1 (cadmium)					
Control	0	6.72	1.8	0.3, 0.5	—
(West Bearskin Lake, MN, USA)	20	6.78	1.9	0.2, 0.7	—
Cadmium	0	6.35	3.2	55, 17	—
(spiked)	20	6.37	3.9	55, 19	—
Study 2 (copper)					
Control	0	7.48	2.0	—	0.5, —
(formulated)	20	7.49	2.5	—	0.5, 0.2
Copper	0	7.18	1.4	—	51, 309
(Keweenaw Waterway, MI, USA)	20	7.52	1.6	—	71, 171

monium and un-ionized ammonia, which occur in an acid–base equilibrium. Our studies found the zeolite treatment to be effective across a wide range of pore-water ammonia concentrations in tests with three species of invertebrates (*H. azteca, C. tentans,* and *L. variegatus*). Zeolite amendments of up to 20% (v/v) were not toxic to these test organisms, except in a single test with *H. azteca.* Zeolite amendments were most successful for reduction of ammonia toxicity to *H. azteca,* the most sensitive of the species tested. Our results, from studies with ammonia-spiked freshwater sediments, suggest that short-term, static toxicity tests maximize differences in pore-water ammonia concentrations and toxicity between sediments receiving zeolite amendments and untreated sediments. The effectiveness of this technique should be confirmed in tests with field-collected sediments with a wide range of ammonia concentrations and other physicochemical characteristics, such as water chemistry, particle-size distribution, and organic carbon content.

The cation-exchange mechanism that removes ammonium from pore water can alter concentrations of major cations in pore water, which can affect the toxicity of both ammonia and metals. Exchange of ammonium from pore water to zeolite results in a net increase in concentrations of other cations in pore water. Greater concentrations of most major cations would be expected to reduce the toxicity of ammonium (i.e., increased sodium and potassium) or reduce the concentration of un-ionized ammonia (lower pH), thereby increasing the intended effect of the zeolite amendment [4,31]. However, increased concentrations of calcium and magnesium (hardness) can reduce the toxicity of cationic metals [33], which could reduce the selectivity of the zeolite treatment. In our studies, zeolite amendments resulted in substantial increases in pore-water hardness in sediments spiked with high concentrations of ammonia and cadmium, but little or no increase in hardness in unspiked natural sediments. These results suggest that changes in concentrations and ratios of pore-water cations are less likely to be a problem in studies with field-collected sediments. Changes in ionic composition of pore water could be minimized by matching the ionic composition of test waters with that of sediment pore waters.

Exchange of cationic metals to zeolite could also interfere with the use of zeolite amendments to selectively reduce ammonia toxicity as part of a sediment TIE protocol, as ammonia and cationic metals commonly co-occur in sediments contaminated by urban and industrial wastes [5]. In our studies, clinoptilolite amendments did not affect the acute toxicity of copper- or cadmium-contaminated sediments and had little or no effect on concentrations of these metals in pore water during toxicity tests. Our results are consistent with the findings of other studies that although zeolites have high exchange capacities for both ammonium and cationic metals, ammonium is exchanged more rapidly than cationic metals [17,19]. This is encouraging evidence that clinoptilolite amendments can be a method to selectively exchange ammonium from sediment pore waters, even in the presence of cationic metals. However, more research is needed to adequately characterize interactions of clinoptilolite with ammonia and cationic metals under a wide range of experimental and environmental conditions.

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